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REVIEW

# Endogenous and Borrowed Proteolytic Activity in the *Borrelia*

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**SUMMARY** The *Borrelia* spp. are tick-borne pathogenic spirochetes that include the agents of Lyme disease and relapsing fever. As part of their life cycle, the spirochetes traffic between the tick vector and the vertebrate host, which requires significant physiological changes and remodeling of their outer membranes and proteome. This crucial proteome resculpting is carried out by a diverse set of proteases, adaptor proteins, and related chaperones. Despite its small genome, *Borrelia burgdorferi* has dedicated a large percentage of its genome to proteolysis, including a full complement of ATP-dependent proteases. Energy-driven proteolysis appears to be an important physiological feature of this dual-life-cycle bacterium. The proteolytic arsenal of *Borrelia* is strategically deployed for disposal of proteins no longer required as they move from one stage to another or are transferred from one host to another. Likewise, the *Borrelia* spp. are systemic organisms that need to break down and move through host tissues and barriers, and so their unique proteolytic resources, both endogenous and borrowed, make movement more feasible. Both the Lyme disease and relapsing fever *Borrelia* spp. bind plasminogen as well as numerous components of the mammalian plasminogen-activating system. This recruitment capacity endows the spirochetes with a borrowed proteolytic competency that can lead to increased invasiveness.

**KEYWORDS** *Borrelia*, borrowed proteolysis, plasminogen, proteases, proteolytic enzymes

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## INTRODUCTION

The spirochete genus *Borrelia* (family *Spirochaetaceae*) was named after the French biologist Amedee Borrel (1). It comprises two main groups, the agents of Lyme borreliosis (LB) and relapsing fever (RF), that share common characteristics, of which 21 are members of LB group and 29 are in the RF group (2). The species constituting the LB group are transmitted by the hard ticks of the family Ixodidae (3), whereas the species in the RF group are transmitted by the soft ticks of the family Argasidae. There are two exceptions, *Borrelia recurrentis*, which is transmitted by the body louse, and *Borrelia miyamotoi*, an RF agent transmitted by the hard tick *Ixodes scapularis* (4). LB and RF *Borrelia* spp. share a set of genetic and biological characteristics that unify these organisms as a group. Namely, all LB and RF *Borrelia* spp. have a similar morphology and an obligate parasitic life cycle and are transmitted between vertebrate hosts by arthropod vectors.

The *Borrelia* spp. have an outer membrane, an inner membrane, and a layer of peptidoglycan within the periplasm. A unique feature of the spirochetes, not just the *Borrelia*, is the presence of endoflagella (or periplasmic flagella) in the periplasmic space, between the outer membrane and the peptidoglycan. Spirochetes are highly motile (5). The *Borrelia* spp. have small genomes with a single linear chromosome and several linear and circular plasmids, a unique feature among eubacteria (6). Another characteristic of *Borrelia* is the abundance of lipoproteins (some in the outer membrane), glycolipids, and cholesteryl glycolipids arranged in clusters (7). For a thorough compilation of the biology of *Borrelia*, please see the recent monograph (8).

The trafficking of *Borrelia* spp. between their vectors and vertebrate hosts requires significant physiological changes, including remodeling of their proteome and outer membranes (9). This is an important feature for the topic of this review, since much of this essential remodeling requires the action of an array of proteases. The proteolytic arsenal of *Borrelia* is strategically deployed for disposal of proteins no longer required as it moves from one stage to another or as it is transferred from one organism to another. Likewise, the *Borrelia* spp. are systemic organisms that need to break down and move through certain tissues and barriers, and their proteolytic resources facilitate this movement.

This review focuses on the endogenous protease arsenal of *Borrelia*, the unique features of this proteolytic resource, as well as the borrowed proteolytic capacity through the use of mammalian proteases—notably, those of the fibrinolytic system. Although we consider the proteolytic activity of both LB and RF organisms, the literature is significantly more skewed with studies of *Borrelia burgdorferi sensu lato*, the agent of Lyme disease (10, 11). As a guide, we have included below an abridged mechanistic description of the major protease types discussed in this review.

### Serine Proteases

Serine proteases are a large and ubiquitous group that use similar mechanisms of action to cleave peptide bonds. In all cases, the side chain hydroxyl of the catalytic serine (Ser) residue is activated to form a nucleophile that attacks the peptide bond. The catalytic serine residue is activated by deprotonation, either in the context of a catalytic triad (Ser, His, and Asp), a mechanism used by the high-temperature requirement proteases (HtrA) and caseinolytic proteases (ClpP), or a catalytic dyad (Ser and Lys), a mechanism used by the Lon proteases, named for the elongated (long) phenotype of cells lacking these key enzymes. Nucleophilic attack by the activated Ser hydroxyl results in cleavage of the peptide bond such that one fragment of the resulting peptide is released whilst the other remains covalently linked to the active site Ser. A water molecule ($H_2O$) is coordinated and activated to break the covalently linked intermediate, thus releasing the second peptide fragment and regenerating the active site Ser (12–15).

### Threonine Proteases

Threonine proteases use the sidechain hydroxyl group of the catalytic threonine (Thr) residue as the nucleophile that attacks the peptide bond of the target polypeptide. However, the mechanism of action of Thr proteases differs from that of the serine proteases in that the Thr hydroxyl group is activated by the $\alpha$-amino group of the same Thr residue rather than a catalytic dyad or triad. This restriction necessitates that the nucleophilic Thr be located at the N terminus of the enzyme. Nucleophilic attack by the activated Thr hydroxyl results in cleavage of the peptide bond such that one fragment of the resulting peptide is released whilst the other remains covalently attached to active site Thr. A water molecule ($H_2O$) is brought to the active site and activated to perform the next step of the reaction, thus releasing the covalently linked second fragment and regenerating the active site Thr (13–15).

### Cysteine Proteases

Cysteine proteases use the thiol group of the active-site cysteine (Cys) as a nucleophile to catalyze peptide bond cleavage. The Cys residue is typically found in a catalytic triad with histidine and aspartic acid, akin to serine proteases, or a catalytic dyad with a histidine residue. In the latter case, it is proposed that the thiol group of the active-site Cys residue is more acidic than the hydroxyl of serine proteases, which obviates the need for the additional aspartic acid residue. The overall reaction mechanism of cysteine proteases is similar to that of serine proteases in that the active site histidine removes a proton to activate the Cys thiol group which, in turn, acts as the nucleophile that attacks and cleaves the peptide bond (13–15).

### Aspartyl Proteases

Aspartyl proteases use two active-site aspartic acid (Asp) residues; one acts as an aspartate (deprotonated version) to coordinate and activate a water molecule as the nucleophile, while the other functions as an aspartic acid (protonated version) to coordinate the carbonyl (C=O) group of the bound polypeptide to make it a better electrophile. The activated hydroxyl (OH) attacks the peptide bond and generates two peptide fragments that are released from the protease without the formation of a covalent intermediate (13–15).

### Metalloproteases

Metalloproteases coordinate metal ions that facilitate cleavage of peptide bonds. The majority of metalloproteases use zinc, with a few using other metals such as cobalt. Metalloproteases use a triad composed of several different amino acids, such as histidine, aspartate, glutamate, arginine, and lysine, to bind the metal ion which, in turn, coordinates a water molecule as the ultimate nucleophile. One typically conserved motif, His-Glu-X-X-His (HEXXH), forms the active site of some metalloproteases, where the two His residues help coordinate the metal ion, which, together with the Glu, helps position the bound water molecule. The Glu residue activates the bound water, thus generating a nucleophilic hydroxyl (OH) that attacks the peptide bond. Cleavage of the peptide bond yields two fragments, neither of which is covalently linked to the enzyme (13–15).

## ENDOGENOUS PROTEASES OF THE *BORRELIA*

### Proteolytic Component of *Borrelia*

The list of 26 annotated proteases of *B. burgdorferi* (Table 1) shows that this bacterium is endowed with a diverse proteolytic arsenal. Of note is the presence of signal peptidases I and II (16). Specifically, there are two signal peptidase I proteins and one signal peptidase II (LspA). LspA has a uniquely important role in the physiology of *B. burgdorferi*, as it cleaves the leader peptides of the large number of lipoproteins that are so critical for this organism. The genomes of relapsing fever *Borrelia hermsii*, an American species, and *Borrelia duttonii*, a sub-Saharan African species, also encode the full complements of these annotated proteases (Table 1). With the exception of HtrA

**TABLE 1** List of annotated proteases of *Borrelia burgdorferi*[a]

| Gene | Locus | Protein | Protease family | Molecular function |
|---|---|---|---|---|
| *lepB* (1) | BB_0031 | LepB signal peptidase I | Serine-type endopeptidase | Cleavage of signal/leader peptides |
| *map 1* | BB_0067 | MAP 1 | Metalloprotease aminopeptidase | Unassigned peptidases (methionyl aminopeptidase I family) |
| *Ap II* | BB_0069 | Aminopeptidase II | Metalloprotease | Putative peptidases |
| *htrA* | BB_0104 | HtrA | Serine endopeptidases | Periplasmic serine protease (Deg family) |
| *map* | BB_0105 | Methionine aminopeptidase | Metalloprotease | Removes the N-terminal methionine from nascent proteins |
| *rseP* | BB_0118 | RseP | Metalloprotease | Putative sigma E protease |
| *cym1* | BB_0228 | Cym1 | Zinc metalloprotease | Putative presequence peptidase |
| *pepF* | BB_0248 | PepF | Serine-type peptidase | Oligopeptidase F |
| *lon1* | BB_0253 | Lon (La) ATP-dependent protease | Serine-type peptidase | ATP-dependent cleavage of peptide bonds with broad specificity; actual substrates in *Borrelia* are not known |
| *lepB* (2) | BB_0263 | LepB signal peptidase I | Serine-type endopeptidase | Cleavage of signal/leader peptides |
| *hslU* | BB_0295 | HslU ATP-dependent chaperone | ClpYQ (HslUV) | ATPase component of the HslUV system; cleavage of peptide bonds with broad specificity |
| *hslV* | BB_0296 | HslV peptidase | Threonine-type endopeptidase activity | Peptidase component of the HslUV system; cleavage of peptide bonds with broad specificity |
| *cptA* | BB_0359 | CptA | Serine-type endopeptidase | Putative carboxypeptidase |
| *apeA* | BB_0366 | ApeA | Putative aspartyl aminopeptidase | Putative aminopeptidase |
| *clpA* | BB_0369 | ClpA ATP-dependent chaperone | | ATP-dependent cleavage of peptide bonds with broad specificity; actual substrates in *Borrelia* are not known |
| *lspA* | BB_0469 | LspA signal peptidase II | Aspartic-type endopeptidase | Prolipoprotein signal peptides, signal peptidase II |
| *pqqL* | BB_0536 | PqqL | Zinc metalloprotease | Putative insulinanse family member |
| Unassigned | BB_0592 | | Glutamate-type endopeptidase | Putative prenyl-processing peptidase family I |
| *lon2* | BB_0613 | Lon 2 (La) ATP-dependent protease | Serine-type endopeptidase | ATP-dependent cleavage of peptide bonds with broad specificity; actual substrates in *Borrelia* are not known |
| *clpP1* | BB_0611 | ClpP peptidase | Serine-type endopeptidase | ATPase component of the ClpXP system; cleavage of peptide bonds with broad specificity; Actual substrates in *Borrelia* are not known |
| *clpX* | BB_0612 | ClpX ATP-dependent chaperone | | Peptidase component of the ClpX/A/C systems; cleavage of peptide bonds with broad specificity |
| *pepX* | BB_0628 | PepX/ApeB | Aspartyl-aminopeptidase | Aminopeptidase family member |
| *clpP2* | BB_0757 | ClpP2 peptidase | Serine-type endopeptidase | Peptidase component of the ClpX/A/C systems; cleavage of peptide bonds with broad specificity |
| *prp* | BB_0779 | Prp | Cysteine-type endopeptidase | Ribosomal-processing cysteine protease |
| *ftsH* | BB_0819 | FtsH ATP-dependent protease | Zinc metalloprotease | Degrades misassembled membrane proteins and cytoplasmic regulatory proteins, including $\sigma^{32}$, LpxC, and $\lambda$CII |
| *clpC* | BB_0834 | ClpC ATP-dependent chaperone | | ATP-dependent cleavage of peptide bonds with broad specificity; actual substrates in *Borrelia* are not known |

[a]The genomes of relapsing fever *Borrelia* species *B. hermsii* and *B. duttonii* carry annotated copies of the genes listed in this table.

(see below), the protease component of all three *Borrelia* species examined is similar. Relapsing fever (RF) and Lyme disease (LD) *Borrelia* spp. are closely related spirochetes but produce different clinical outcomes in animals and humans based on their respective abilities to cause a recurrent spirochetemia or an invasion of multiple organs. Based on our examination of the annotated proteases of the three representative species, it is not possible to conclude that their proteolytic components could contribute to these differences.

The first attempts at detecting proteolytic activity in *B. burgdorferi* utilized collagen (II and IV) and gelatin as the substrates. Collagenolytic activity was detected using spirochete lysates (17). Since then, other proteases have been studied in greater detail. Not surprisingly, despite its relatively small genome size, *B. burgdorferi* has dedicated a larger percentage of its genome to proteolysis than bacteria with larger genomes (Table 2). That *B. burgdorferi* has a full complement of the ATP-dependent proteases (Table 2) indicates that energy-driven proteolysis is a very important physiological feature of this dual-life-cycle bacterium.

The energy-dependent AAA+ (ATPases associated with diverse cellular activities) proteases degrade damaged, misfolded, and surplus proteins and remove key regulatory proteins following major life cycle transitions and growth condition changes. The ATP-dependent proteases that drive these processes must be highly specific to avoid untimely degradation of essential proteins. *B. burgdorferi* contains six ATP-dependent proteases: ClpXP, ClpAP, ClpCP, HslUV, FtsH, and Lon (Tables 1 and 2).

Energy-dependent proteases are composed of an AAA+ component and a peptidase component. These two components can be encoded by the same gene, as is the case for Lon (Fig. 1, left) and for FstH, or by two distinct genes, one encoding the AAA+ chaperone/unfoldase (ClpX, ClpA, and ClpC) and the other encoding the ClpP peptidases (Fig. 1, right). Furthermore, the unfoldases belong to the Hsp100/Clp family of proteins and contain one (in the case of ClpX) or two (in the case of ClpA, ClpB, or ClpC) ATPase domains. All of these ATP-fueled nanomachines can unfold and degrade folded proteins. The active AAA+ protease form ring-shaped oligomeric assemblies (Fig. 1) with a central pore leading to the sequestered peptidase chamber (18). While most Gram-negative bacteria contain ClpXP and ClpAP, Gram-positive bacteria typically contain some combination of ClpXP, ClpCP, and ClpEP. For instance, *Staphylococcus aureus* contains ClpXP and ClpCP, where ClpCP is more effective at degrading damaged proteins than ClpXP (19). Curiously, *B. burgdorferi* has all three Clp proteases (ClpAP, ClpXP, and ClpCP) and carries two ClpP peptidases, ClpP1 and ClpP2 (Table 2).

HslUV, also known as ClpYQ, is formed by a union of the dodecameric peptidase HslV and its hexameric, ATP-dependent partner, HslU. HslUV is an AAA+ protease found in certain bacteria, including *B. burgdorferi* (Table 2), and in the mitochondria of some lower eukaryotes, such as trypanosomatids, and shares sequence, structural, and mechanistic similarities with the more sophisticated 20S proteosome. The HslU unfoldase regulates the proteolytic activity of the HslV peptidase and is responsible for the substrate specificity of the protease (20, 21). Moreover, HslUV and ClpXP have been implicated in the regulation of the SOS response, polysaccharide synthesis, RNA metabolism, and symbiotic nitrogen fixation (22). The FtsH and Lon proteases of *B. burgdorferi* are considered below.

### Amino and Carboxypeptidases of *B. burgdorferi*

An aminopeptidase from *B. burgdorferi* was shown to have aminopeptidolytic activity in spirochete lysates. The purified protein is a 300-kDa hexamer formed by 50-kDa monomers. The enzyme was identified as zinc-dependent aminopeptidase II (BB0069) with significant identity to the M29/T family of metallopeptidases (23).

CtpA, a carboxyl-terminal protease in *B. burgdorferi* is involved in the processing of P13 and BB0323, and *ctpA* inactivation has a pleiotropic effect on *Borrelia* protein synthesis (24). Site-directed mutagenesis to alter CtpA cleavage sites of the outer membrane (OM) porin P13 resulted in ectopic expression of a C-terminally truncated P13.

**TABLE 2** ATP-dependent proteases of selected bacterial and other species

| Species | No. of encoded proteins | Lon | ClpX | ClpP | ClpA | ClpC | FtsH | HslUV |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | 4289 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *B. subtilis* | 4100 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *Mycobacterium tuberculosis* | 3918 | ✓ | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | ✓ |
| *Synechocystis* | 3169 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × |
| *Deinococcus radiodurans* | 2580 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × |
| *Thermotoga maritima* | 1846 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *H. influenzae* | 1709 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ |
| *Aquifex aeolicus* | 1522 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *H. pylori* 26695 | 1566 | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Treponema pallidum* | 1031 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ? |
| *Chlamydia pneumoniae* | 1052 | ✓ | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | × |
| *Chlamydia trachomatis* | 894 | ✓ | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | × |
| *B. burgdorferi* | 850 | ✓ (Lon1 and Lon2) | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | ✓ |
| *B. hermsii* | 827 | ✓ (Lon1 and Lon2) | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | ✓ |
| *B. duttonii* | 850 | ✓ (Lon1 and Lon2) | ✓ | ✓ (ClpP1 and ClpP2) | ✓ | ✓ | ✓ | ✓ |
| *Rickettsia prowazekii* | 834 | ✓ | ✓ | ✓ | × | × | ✓ | ✓ |
| *Mycoplasma pneumoniae* | 677 | ✓ | × | × | × | × | ✓ | × |
| *Mycoplasma genitalium* | 467 | ✓ | × | × | × | × | ✓ | × |
| *N. gonorrhoeae* | 2030 | ✓ | ✓ | ✓ | ✓ | × | ✓ | ? |
| *Caulobacter crescentus* | 3884 | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Y. pestis* | 3873 | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Streptococcus pyogenes* | 1680 | ✓ | ✓ | ✓ | × | ✓ | ✓ | ? |
| *Staphylococcus epidermidis* | 2326 | ✓ | ✓ | ✓ | × | ✓ | ✓ | ✓ |
| *Listeria monocytogenes* | 2930 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *Agrobacterium tumefaciens* | 5163 | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Clostridium tetani* | 2692 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ? |
| *Arabidopsis thaliana* | 29085 | ✓ | ✓ | ✓ | ✓ limited homology | ✓ | ✓ | ✓ |
| *Homo sapiens* | ~21000 | ✓ | ✓ | ✓ | ✓ limited homology | × | ✓ | × |

Additionally, a lower-molecular-weight variant of surface lipoprotein OspC was mislocalized to the periplasm. Further investigation revealed that the OspC variant resulted from C-terminal proteolysis by CtpA. These findings emphasized that the CtpA of *B. burgdorferi* is not specific for targeting proteins that lack structurally constrained C termini, as proteolysis appears to occur independently of a specific peptide recognition sequence (25).

### FtsH of *B. burgdorferi*

Molecular chaperones ensure correct protein function by supervising protein folding and shuttling proteins to their final destinations. Misfolded, aberrant, and unwanted proteins, on the other hand, are targeted for proteolytic degradation. The membrane-anchored, 71-kDa ATP-dependent zinc metalloprotease FtsH fulfils these functions in bacteria, mitochondria, and chloroplasts, where it degrades cytoplasmic and membrane proteins. While FtsH is essential for the viability of *Escherichia coli*, *Bradyrhizobium japonicum*, and *Helicobacter pylori*, in other bacteria, it is important for cell viability only during stress conditions and stationary-phase growth. Known FtsH substrates include the heat shock response sigma factor $\sigma^{32}$ and the lipid A biosynthesis enzyme LpxC. In fact, absence of FtsH perturbs the balance of lipopolysaccharide and phospholipid synthesis, resulting in cell death. The SecYEG translocon, which facilitates protein transport across the inner membrane, is also subject to proteolytic regulation by FtsH. When not in complex with SecE and SecG, SecY is recognized and degraded by FtsH. Additionally, FtsH degrades KdtA, a transferase required for the biosynthesis of oligosaccharides (26, 27).

The FtsH of *B. burgdorferi* (FtsHBb) has the ATP-binding consensus sequences and the conserved proteolytic HEAGH (HEXXH) motif, suggesting it functions like the canonical FtsH from *E. coli*. FtsH degrades misfolded cytosolic and membrane proteins and possess the capacity to function as a chaperone (28). The canonical *E. coli* FtsH

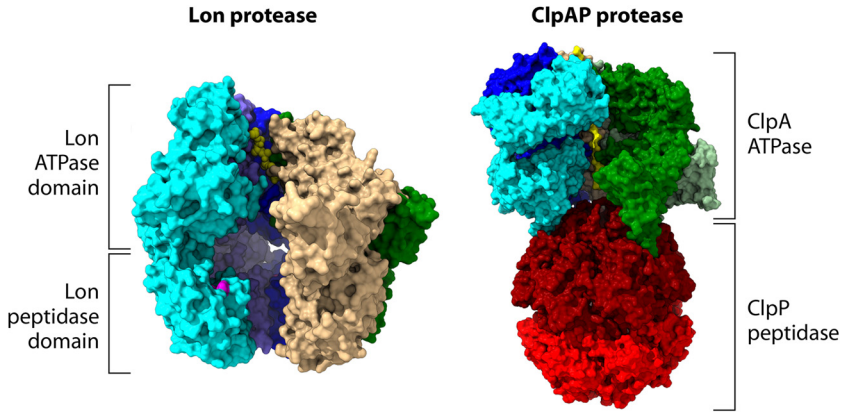


**FIG 1** Architectural arrangements of AAA+ proteases. (Left) The structural model of Lon protease hexamer in the active substrate-engaged state is shown (PDB 6ON2). Each Lon monomer contains both the ATPase and the peptidase domains. In this depiction, one of the six Lon protomers is omitted to highlight the bound substrate (yellow), staircase arrangement of the active substrate engaged enzyme, and the peptidase active-site Ser-Lys dyad (purple). (Right) The structural model of the ClpAP protease in the active substrate-engaged state is shown (PDB 6W1Z). The ClpA ATPase forms a homohexamer that sets on top of the assembled ClpP peptidase, which is composed of two stacked heptamers (dark red and red, respectively). In this depiction, one of the six ClpA protomers is omitted to highlight the two AAA+ domains and the bound substrate (yellow).

associates with modulators HflC/HflK for oligomerization (28–31). The presence of the three proteins (HflC, HflK, and FtsH) in lipid rafts of *B. burgdorferi* strongly indicates that the complex carries out its activity in these microdomains (32, 33). The genes for the FtsH protease and its modulators, HflK and HflC, were deleted, with interesting and unexpected results. FtsH depletion (but not overproduction) in *B. burgdorferi* resulted in membrane deformation and cell death. In contrast, deletion of the FtsH modulators HflK and HflC (ΔHflK/C) did not alter morphology, growth rate, growth under stress conditions, or infectivity (26).

### Lon Proteases of *B. burgdorferi* Are Unusual in the Eubacteria

As a member of the AAA+ protease family, Lon can be found in bacteria, archaea, and eukaryotes. Extensively studied in the context of bacterial proteolysis, the ATP-dependent Lon protease regulates a variety of cellular processes, such as capsule synthesis, genetic competence, cell motility, cell division, DNA replication, heat shock response, and pathogenesis. Lon is also thought to be responsible for degrading approximately 50% of all misfolded proteins in *E. coli*, highlighting its integral role in preserving cell viability. As a proteolytic regulator, Lon degrades various native regulatory proteins that modulate major cellular processes. These include the following: HU$\beta$, the DNA-binding histone-like protein that plays a role in DNA replication and gene regulation; SulA, a critical cell division protein; RcsA, the capsule synthesis regulator; CcdA, an antitoxin that is part of the F plasmid-based killing system; IbpA, a heat shock protein; SoxS, a transcription activator that plays an important role in removing reactive oxygen species; SwrA, the master regulator of flagellar biosynthesis and cell motility; and transfer-messenger RNA (tmRNA)-tagged proteins (34–40). A common feature among proteases, Lon is assisted by adaptor proteins (41) that enhance its activity and fine tune its substrate specificity. Two such Lon-specific adaptors have been identified: SmiA, which mediates the degradation of SwrA in *Bacillus subtilis*, and HspQ (39, 42). As one of the most intriguing adaptor molecules, HspQ is itself a Lon substrate while also acting as an allosteric activator of the protease. Lon-mediated engagement and degradation of HspQ enhances the proteolysis of several native regulatory substrates, such as YmoA (*Yersinia* modulating protein A), Y0390, Fur (ferric uptake regulator), and RsuA (ribosomal small subunit pseudouridine synthase A) (42, 43). Lon protease of *Salmonella* degrades the heat-stable nucleoid structuring protein (H-NS),

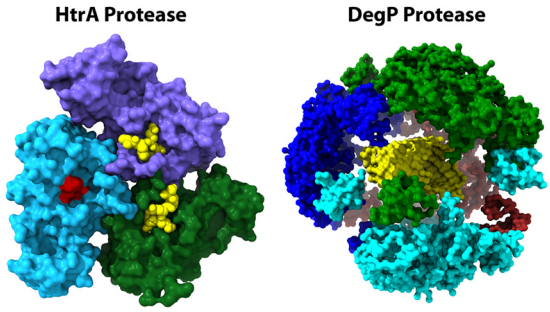


**FIG 2** Architectural arrangements of HtrA and DegP proteases. (Left) The structural model of HtrA protease trimer in the active peptide-bound state is shown (PDB 2Z9I). Each HtrA monomer is shown in a different color, with two monomers containing a bound autoproteolytic peptide (yellow) to the active site. The third monomer is shown without the bound peptide to highlight the active-site Ser-His-Glu catalytic triad (red). (Right) A structural model of the DegP protease dodecamer in the substrate-bound state (PDB 4A8D) is shown. The DegP chaperone forms a cage-like structure composed of 4 trimers, each depicted in a different color, with the bound substrate (outer membrane protein [OMP]; yellow) captured inside the dodecameric cage.

unleashing a complex chain of events that enables the pathogen to express foreign virulence genes during infection (43).

*B. burgdorferi* harbors two Lon proteases, Lon-1 and Lon-2 (44), which is a rare but not unique occurrence. The first evidence for the presence of the *lon* gene of *B. burgdorferi* yielded a protein of 806 amino acids with substantial sequence identity to other bacterial Lon proteases. Transcriptional upregulation of *lon-1* follows exposure to blood *in vitro* (45). The *lon-2* gene is immediately downstream of ATP-dependent proteases *clpP* and *clpX*, as is in *E. coli lon*. Lon-1 and Lon-2 of *B. burgdorferi* cluster separately in the N-terminal substrate-binding domains, and this could reflect differences in substrate specificity. Recombinant Lon-1 had the properties of an ATP-dependent chaperone protease, with a catalytically active serine-lysine dyad, that has caseinolytic activity *in vitro*. However, the recombinant Lon-1 could not degrade an ssrA-tagged substrate (41, 46). Moreover, *lon-1* did not complement an *E. coli lon* mutant, while Lon-2 corrected two characteristic phenotypes of the *lon*-mutant. Lon-2 functions in a manner consistent with canonical Lon, engaged in cellular homeostasis. Thus, Lon-1, as a result of its blood induction and as a unique feature of *Borrelia*, is important during the transition from the arthropod vector to the mammalian host.

The role of the two *Borrelia* Lon proteases in murine infection indicate that Lon-1 plays a critical role for the infection of *B. burgdorferi* (47). A *lon-1* deletion mutant was attenuated in mice and displayed other characteristics such as growth defects in BSK-II medium and resistance to osmotic stress. Production of BosR, RpoS, and OspC increased in the mutant, suggesting that one or all of these regulatory proteins may be Lon-1 substrates. A catalytic-site Ser-to-Ala mutant did not infect mice, suggesting that the proteolytic activity of Lon-1 is essential for infection (47, 48). A *lon-2* deletion mutant was attenuated in mice but did not have a growth defect in culture (48). This mutant showed resistance to osmotic stress, and protein levels of RpoS and OspC were decreased. The finding that neither *lon1* nor *lon2* is essential may indicate that their activities are synergistic and that, despite their biochemical differences, one can compensate, at least partially, in the absence of the other.

### HtrA of *Borrelia*, a New Strategy for Pathogenesis

The HtrA family of serine proteases can be found in all cells, from prokaryotes to primates. Unifying features of this family are the trimeric structure and the proteolytic domain catalytic triad composed of Ser-His-Asp (Fig. 2). These proteases also have one or two C-terminal PDZ domains that mediate protein-protein interactions (49).

*E. coli* DegP was the first periplasmic HtrA protease characterized (50, 51). It functions as a protease to degrade misfolded proteins and as a chaperone during the protein-folding stress response (51). As a chaperone, DegP protects proteins from

degradation in the periplasm, and as a protease, it degrades selected substrates (52). The DegP trimer can auto-oligomerize after binding a misfolded or partially folded substrate, forming a macromolecular cage-like structure (Fig. 2B) that functions as a chaperone to protect the trafficking of outer membrane proteins through the periplasm (53, 54).

The first identification of the HtrA homolog (BhpA) in the genus *Borrelia* was made in two species of relapsing fever spirochetes, *B. hermsii* and *B. turicatae*. Although most relapsing fever organisms have the *bhpA* gene, it is not shared with *B. burgdorferi* (55). Recombinant BhpA protein degrades $\beta$-casein and is transcribed at all growth temperatures *in vitro* but at much higher levels during *B. hermsii* infection in mice. A gene encoding BtpA, an HtrA family protease, is present in *B. turicatae* (55). Transcriptional analyses in *B. turicatae* revealed that *btpA* was expressed as part of an operon. Indeed, it was demonstrated that BtpA and proteins encoded by two adjacent genes were produced in response to culture at mammalian body temperature. Mutants of *btpA* exhibit no growth defects in response to heat shock but are more sensitive to oxidative stress. The fact that the *btpA* mutant is infectious in the murine model indicates that its function is not required for pathogenesis (56). A phylogenetic analysis showed that *B. hermsii* BhpA is the homolog of BhtA of *B. turicatae* but not of *B. burgdorferi* HtrA (48). Our own phylogenetic analysis agreed with these results (50).

*B. burgdorferi* harbors a gene for a single HtrA protease (HtrABb) on its chromosome that shares ~40% amino acid homology with DegP from *E. coli*. This protease has been the subject of several studies that disclosed some unique features as well as a possible role in pathogenesis. HtrABb processes a conserved protein substrate, BB0323, into N- and C-terminal fragments that are biologically active and supports spirochete growth (57, 58). The cleavage of BB0323 has critical functional implications in the spirochetal life cycle, as the N-terminal fragment is important for cell fission and the C-terminal LysM domain fragment is essential for mammalian infection. Biochemical studies showed that HtrABb, like DegP, has the trimer as its fundamental unit (Fig. 2A). Recombinant HtrABb degrades casein, while its catalytic serine mutant (HtrABbS198A) does not (59). Despite its homology to DegP, HtrABb does not complement an *E. coli degP* deletion mutant. Two additional HtrABb substrates, basic membrane protein D (BmpD/BB0385) and chemotaxis signal transduction phosphatase CheX (BB0671), have been identified. BmpD is an important immunogenic adhesin expressed in infected patients, and CheX is a crucial protein for motility (59). HtrABb may function in regulating outer membrane lipoproteins and in modulating the chemotactic response of *B. burgdorferi*. Proteolytic activity of HtrABb is inhibited by micromolar concentrations of zinc preferentially over copper and manganese (60). FliD, a flagellar cap protein, promotes flagellin (FlaB) polymerization and filament growth within the periplasm. Deletion of *fliD* leads to an accumulation of unpolymerized FlaB, which is degraded by HtrA in the periplasm (61). HtrABb-dependent proteolysis of FlaB and CheX, as well as the defect on swarm assays, suggests that HtrABb plays an important role in regulating *B. burgdorferi* motility (5, 61).

An HtrABb overexpression approach identified outer membrane protein P66 as a substrate. Both P66 and HtrA partitioned into detergent-resistant membranes, which contain cholesterol-glycolipid-rich membrane regions known as lipid rafts. This agreed with previous work which showed that HtrABb and p66 are constituents of *B. burgdorferi* outer membrane vesicles (62). Their colocalization establishes that they could interact efficiently, and their protease/substrate relationship provides functional relevance to this interaction. The overexpresser strain showed reduced levels of *p66* transcript, indicating that HtrABb-mediated regulation of p66 may occur at multiple levels. There is increasing evidence that HtrABb could be located in a functional form outside the periplasm (63). An HtrABb-overexpressing strain of *B. burgdorferi* (A3HtrAOE) revealed that protein levels of P66 were reduced compared to that in wild-type *B. burgdorferi*, consistent with its being a substrate of this protease. In addition, Hbb, a *p66*-DNA-binding transcription factor (64), was specifically degraded by HtrABb, providing further

evidence for a role for both in the regulation of P66. P66 is a very important outer surface protein for *B. burgdorferi*, as it functions as both an adhesin and a porin (65–67).

The A3HtrAOE strain displayed reduced motility in swarm assays, indicating a link between overexpression of HtrABb and its enzymatic specificity for P66. However, the Δ*p66* strain did not have reduced motility in the swarm assays, negating a role for this protein. The proteomics analyses also showed that overexpression of HtrABb had an impact in the production of the cellular levels of three glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glycerol-3-phosphate dehydrogenase (GPDH), and glycerol kinase (GK), and an enzyme involved in carbohydrate metabolism, diphosphate-fructose-6-phosphate 1-phosphotransferase. Consistent with the reduced protein levels of these glycolytic enzymes, A3HtrAOE was also deficient in production of pyruvate. These experiments suggested a role for HtrABb in regulating the metabolic activity of *B. burgdorferi* (68).

Lmp1, a surface-exposed protein involved in various functions in spirochete infectivity, is another HtrABb substrate (69). HtrABb degrades Lmp1 into polypeptide fragments that are essential for transmission of *B. burgdorferi* from ticks to mammals (69). That Lyme disease patients, as well as mice experimentally infected with *B. burgdorferi*, produce antibodies to HtrABb (59, 70, 71) prompted an attempt to determine whether recombinant HtrABb could be used as a vaccine, but it failed to protect the mice after a challenge with the organisms (72).

Recent studies succeeded in creating a targeted deletion of HtrABb, suggesting a nonessential role for this protease in microbial viability. However, the mutant displayed growth, morphological, and structural defects during cultivation at 37°C, confirming a high-temperature requirement for protease activation and function. Moreover, HtrABb-deficient spirochetes were unable to establish infection in mice. Earlier studies confirmed HtrA-mediated proteolytic processing of BB0323 (discussed above), the lack of which likely contributed to the inability of the mutant strain to survive in a mammalian host (73).

Canonical HtrA is a periplasmic protease, but there is enough evidence to support proteolytic activity in other bacterial cell compartments as well as in outer membranes. The location of HtrABb outside the canonical periplasm was established through proteinase K studies that demonstrated its surface exposure. Using sera from patients with early and late Lyme disease, this study also established that HtrABb is immunogenic, thus confirming its expression during the disease process. Additional HtrABb extracellular substrates have been identified in the tissues preferred by *B. burgdorferi*. Aggrecan, a cartilage-specific proteoglycan, is an HtrABb substrate (71) as are proteoglycans of the extracellular matrix (ECM) and fibronectin. Fragments derived from the degradation of fibronectin amplify the inflammatory process, thus contributing to the pathogenesis of Lyme disease. Exposure of chondrocytes to recombinant HtrABb leads to the production of proinflammatory cytokines and chemokines that are well-known participants in the Lyme arthritis milieu. Therefore, HtrABb is thought to actively participate in dissemination and in tissue damage due to inflammation triggered by Lyme disease (70).

We found that HtrABb was one of a group of proteins detected by mass spectrometry in isolated *B. burgdorferi* vesicles (32, 33). HtrABb exists in both membrane-bound and soluble forms and is detectable in conditioned medium (59, 70). Indeed, exo-periplasmic location and activity for HtrA have been well established in other pathogens such as *Campylobacter jejuni* (74) and for extracellular secretion and degradation of E-cadherin by *Helicobacter pylori* (75, 76). The same is true for secreted HtrA proteases from other Gram-negative pathogens in cleaving junctional proteins (75, 76). Extracellular proteolysis by secreted HtrA is thought to augment the invasiveness of pathogenic bacteria by degrading host ECM components and cellular junctional molecules. This topic is considered in a review where the proteolytic activities of secreted HtrA from both Gram-positive and Gram-negative pathogens promote invasiveness and cell damage and thus constitutes a novel mechanism for pathogenesis (77). It is

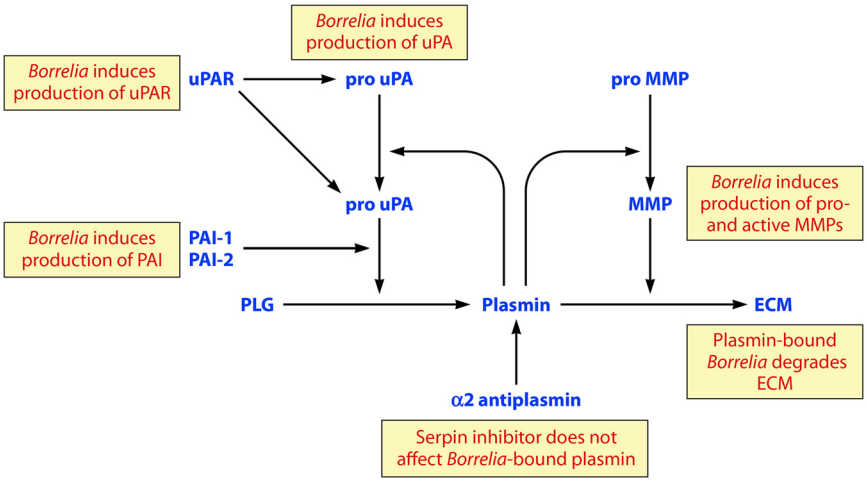


**FIG 3** Mammalian plasminogen activation system indicating all of its components that interact with *Borrelia* species.

proposed that HtrA export is a newly acquired strategy used by an increasing number of bacterial pathogens.

## *BORRELIA* AND BORROWED PROTEOLYSIS

This section considers the vast literature on the utilization of host proteases by *Borrelia*. Research into borrowed proteolysis has yielded valuable insights into spirochetal biology.

### Plasminogen Activation System

The plasminogen activation system (PAS) is a highly controlled system for dissolving fibrin clots (thrombi) that has been implicated in a diverse set of normal and pathological processes. Its central effector element, plasminogen (PLG), is a zymogen initially expressed in humans as an 810-amino-acid polypeptide that is subsequently modified to a 791-amino-acid mature protein (78). PLG circulates in the blood and tissue fluids at a concentration of approximately 2 $\mu$M (79) and is activated enzymatically to form active plasmin through cleavage of its $Arg^{561}$-$Val^{562}$ bond. Plasmin has a broad trypsin-like range of specificity, enabling it to degrade a wide variety of substrates in addition to its canonical role in thrombolysis (Fig. 3).

This powerful source of proteolysis, due to its high concentration in circulation, is tightly controlled by a set of two PLG activators (PAs), originally identified in urine and tissue extracts and that have been classified as belonging to distinct classes. Urokinase-type plasminogen activator (uPA) is a 411-amino-acid zymogen (pro-uPA) that is activated by plasmin cleavage at $Lys^{158}$-$Ile^{159}$ to generate uPA, a two-chain enzyme connected by a disulfide bond. Tissue-type plasminogen activator (tPA) is a proteolytically active, 527-amino-acid single-chain molecule that is also processed by plasmin into a two-chain form, which is substantially more active than the single-chain form. uPA and tPA are alike in that they are activated by plasmin but have mainly divergent roles, with uPA being linked to inflammation, wound healing, tissue remodeling, metastasis, and invasion (80–82).

Unchecked plasmin elicits a cascade of proteolytic events that can lead to degradation of ECM, diseases of the vasculature, and cancer metastasis (83). To prevent uncontrolled plasmin activity and pathological tissue damage, a system of protease inhibitors, acting either at the plasmin level or at the PA level, is constitutively expressed. At the plasmin level, alpha2-antiplasmin ($\alpha$2-AP), a 70-kDa serpin, inhibits plasmin very rapidly, forming a 1:1 stoichiometric complex. At the PA level, serpin plasminogen activator inhibitor (PAI-1) and, to a lesser extent, PAI-2, PAI-3, and protease nexin inhibit uPA and thus plasmin, by binding to the uPA active site (84, 85).

Urokinase plasminogen activator receptor (uPAR; CD87), a glycosylphosphatidylinositol (GPI)-anchored 55- to 60-kDa protein (86–89) first identified in monocytes (90, 91), binds to uPA, pro-uPA, and the ECM glycoprotein vitronectin (92). Plasmin bound to uPAR is protected from inactivation by plasminogen activator inhibitors (PAI) and $\alpha$2-AP (unbound plasmin is efficiently inactivated by PAIs). In addition, plasmin bound to fibrin is several orders of magnitude more resistant to inactivation by $\alpha$2-AP (78). In both normal and pathological settings, cell-associated plasmin acquisition is believed to be a product of cell-associated uPA activation of PLG. A keynote occurrence in this process is the interaction between uPA and its high-affinity receptor, uPAR (86, 87, 89). The focus of uPA and, subsequently, plasmin generation on the cellular leading edge is thought to be the means by which inflammatory and cancer cells (uPAR is overexpressed in metastatic cancers of various types) degrade ECM and migrate from one anatomical site to another. This supports its role as a central player in mediating ECM degradation during inflammation and cancer progression (93).

**Plasmin(ogen) Binding and Invasion by Bacteria**

In addition to innate genome-encoded proteases, there is an expanding collection of bacteria (both Gram positive and Gram negative) that acquire proteolytic activity by borrowing host PAS components. This occurs through binding of five PLG kringle structures to exposed lysine residues on the bacterial surface (94, 95). Once bound, the PLG can be activated to plasmin, either by native PAs or by further interaction with the host PAS through binding of uPA or, less commonly, tPA. The binding of plasmin to lysine residues on the bacterial surface is inhibited by competition with the lysine analogs $\varepsilon$-aminocaproic acid (EACA) and tranexamic acid, confirming its lysine specificity (96). At the plasmin level, lysine binding is inhibited by the presence of the physiological inhibitor $\alpha$2-AP; however, bound plasmin is protected from deactivation by $\alpha$2-AP (79, 97–99). Supplementary plasmin activity inhibition can also be achieved by nonspecific plasma serpin $\alpha$2-macroglobulin (100). Interestingly, the iron-binding milk lactoferrin blocks PLG conversion to plasmin by binding to PLG (101).

PLG-binding bacteria can be divided into two basic categories: those that express endogenous PAs and those that do not. Among those that produce their own PAs are *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Yersinia pestis* (streptokinase, staphylokinase, and plasminogen activator Pla, respectively) (102–107). Streptokinase and staphylokinase are not proteases but form a 1:1 complex with PLG and plasmin, exposing the active site of plasmin and causing conformational changes leading to increased specificity and efficiency for both (41, 108, 109). In contrast to streptokinase and staphylokinase, Pla is a protease and functions in a manner similar to that of uPA and tPA by activating PLG-to-plasmin conversion through proteolytic cleavage of the $Arg^{560}$-$Val^{561}$ peptide bond (110). The bacteria that borrow PAs from the host in order to activate bound PLG include *Haemophilus influenzae*, *Moraxella catarrhalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* (111, 112), *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Helicobacter pylori* (113, 114), *Salmonella enterica* serovar Typhimurium (110), *Escherichia coli* (115, 116), and *Borrelia* spirochetes, including *B. burgdorferi sensu stricto* (3, 117), *B. garinii*, *B. afzelii* (*sensu lato*), *B. recurrentis* (118), *B. hermsii* (119), and other *Borrelia* spp. (120).

**Plasmin(ogen) Binding by *Borrelia***

The expanding literature on *Borrelia* and the PAS justifies its treatment as a subject on its own. For that reason, the focus of this review section will be on the interaction with the host PAS by *B. burgdorferi* and related *Borrelia* species only; for reviews of other bacteria and PLG, see reference 110. Figure 3 shows the high level of utilization of the PAS by *Borrelia*. It can be appreciated that all the components of the PAS can interact with these organisms. The affinity of *Borrelia* for PLG is firmly grounded on the unique aspects of its biology. The genome of *B. burgdorferi* is A+T rich (72%) (6), as are the genomes of two RF species, *B. parkeri* (121) and *B. hermsii* (122). The most frequently used codon is AAA, which, along with AAU, codes for lysine, the amino acid

associated with binding to PLG. Not surprisingly, the lysine content of *B. burgdorferi* is 10.2% (6). The ubiquity of lysine residues in *Borrelia* is not only the major factor influencing binding of these organisms to PLG but is also contributory to binding to negatively charged molecules such as proteoglycans and others. Although outside this review, the genomes of *Staphylococcus aureus* and species of *Streptococcus*, also notable for PLG binding, are similarly A+T rich.

*B. burgdorferi*, *B. garinii*, *B. afzelii*, *B. spielmanii*, and *B. miyamotoi*, the major agents of Lyme disease and miyamotoi disease in North America, Europe (117, 123–126), and Asia (4, 127–130), are transmitted by hard ticks of the genus *Ixodes*. These pathogens spread hematogenously from the initial site of tick attachment to colonize a variety of tissues and organs, causing joint swelling, complications of the heart and nervous systems, and acrodermatitis chronica atrophicans, a late skin manifestation (131–133). The penetration of tissue barriers (capillary endothelium, ECM, and basement membranes) by the spirochete is a requirement to establish systemic Lyme disease infection and necessitates the proteolytic breakdown of these aforementioned ECM components (134, 135).

Although *Borrelia* spp. have been shown to adhere to a large diversity of mammalian and tick cells, mostly under laboratory conditions (136), the preferred site, as observed *in vivo*, is in the ECM and connective tissue (137–143).

In addition to *B. burgdorferi* (*sensu stricto*), the binding of PLG to spirochetal outer membrane has been documented to occur in a variety of other *Borrelia* species, including agents of Lyme disease in Europe, *B. garinii* and *B. afzelii* (*sensu lato*), and in North America/Asia (144, 145). Relapsing fever *Borrelia* species *B. hermsii* (119, 146), *B. coriaceae*, *B. parkeri*, *B. turicatae* (144), and *B. crocidurae* (147) also have been shown to bind plasmin(ogen). In addition to that of PLG, binding of uPA to the outer membrane has also been documented in *B. burgdorferi* as well as in other *Borrelia* species. The binding was diffuse in species other than *B. burgdorferi* but was localized to the spirochete terminus. In mice, uPA-coated *B. burgdorferi* exhibited increased infectivity (144, 148). The PAS also has a role in heart and brain invasion by relapsing fever *Borrelia*, resulting in organ injury (143, 147). RF spirochetes utilize the PAS for organ invasion, as has been shown in *in vivo* studies.

### Substrates Degraded by *Borrelia* with Bound Plasmin

Degradation of chromogenic substrate S-2251 has been utilized to identify plasmin-bound proteolytic activity in bacterial species, including *Borrelia* (142, 149, 150). The plasmin in turn can degrade glycoprotein ECM components such as fibronectin (149), laminin, and vitronectin but not collagen (148, 151). *B. burgdorferi* complexed with plasmin was shown to degrade an *in vitro* ECM model (Table 3) (142). Spirochetes with bound PLG or uPA alone as well as spirochetes with bound plasmin in the presence of serine protease inhibitor aprotinin did not show any significant ECM proteolysis (151, 152). Such a process occurring *in vivo* would represent a powerful mechanism for the spirochete to disseminate from the bloodstream to colonize distant tissues/organ sites in the host.

### *Borrelia* and Plasminogen Receptors

The localization of plasmin(ogen) to the *B. burgdorferi* outer membrane has led to investigations to identify receptor proteins accessible for PLG binding. Among the many reported *B. burgdorferi* PLG-binding proteins, several have been characterized (Table 3). Outer membrane lipoprotein OspA was implicated early on as a spirochetal receptor for PLG (142, 149, 152). It is now accepted that OspA, under the control of alternative sigma factor RpoS (153), is downregulated soon after *B. burgdorferi* is transmitted to the mammalian host (154), throwing into question its biological relevance for plasmin-mediated pathogenesis in mammals. OspA may still play an important role in PLG-mediated spirochete dissemination in the tick (155). On the other hand, outer membrane lipoprotein OspC, a 21-kDa lipoprotein critical for infection in mice (156), has been proposed as a biologically relevant PLG receptor (157, 158). In strains where it is highly expressed, OspC binds PLG, and the two molecules colocalize in the outer membrane, which facilitates its

**TABLE 3** *Borrelia* ligands for plasminogen

| *Borrelia* PLG ligand | Species | Disease | PLG ligand (kDA) | Reference(s) |
|---|---|---|---|---|
| OspA[a] | *B. burgdorferi* | LD[b] | 30 | 142, 149 |
| OspC | *B. burgdorferi* | LD | 21 | 158 |
| Enolase | *B. burgdorferi* | LD | 47 | 170, 171, 172 |
| BpcA | *B. parkeri*, *B. turicatae* | RF[c] | 17 | 164 |
| ErpP[d] (CRASP[e]-3) | *B. burgdorferi* | LD | 19 | 159 |
| ErpC (CRASP-4) | *B. burgdorferi* | LD | 60 | 159 |
| ErpA (CRASP-5) | *B. burgdorferi* | LD | 18 | 159 |
| CRASP-1 (CspA) | *B. burgdorferi*, *B. afzelii*, *B. spielmanii* | LD | 28 | 120, 161, 192 |
| BhCRASP1/FH | *B. hermsii* | RF | 21.5 | 163 |
| BBA70 | *B. burgdorferi* | LD | ~30 | 150 |
| CbiA | *B. miyamotoi* | MD[f] | 21 | 145 |
| 70-kDa BPBP | *B. burgdorferi* | LD | 70 | 152, 175 |
| HcpA | *B. recurrentis* | RF | | 118 |
| FhbA[g] | *B. hermsii* | RF | 20.5 | 119 |

[a]OspA, outer surface protein A.
[b]LD, Lyme disease.
[c]RF, relapsing fever.
[d]Erp, OspF/E-related protein.
[e]CRASP, complement regulator-acquiring surface protein.
[f]MD, miyamotoi disease.
[g]FhbA, factor H binding protein A.

conversion to proteolytically active plasmin with the addition of exogenous uPA. This colocalization is not observed in an OspC knockout (158). The lack of significant PLG binding in an OspC-deficient *B. burgdorferi* strain is seemingly in contrast with the literature, since there has been evidence given for alternative PLG-binding sites in several studies. For example, outer membrane ErpP proteins (OspE/F-related), also known as complement regulator-acquiring surface protein (CRASP)-3, CRASP-4 (ErpC), and CRASP-5 (ErpA), have also been reported to bind PLG where it is converted to plasmin, possibly increasing the spirochetes' ability to disseminate through proteolysis of the host ECM (159). Members of the CRASP family also bind complement regulator factor H, FHL-1 (factor H-like protein), and complement factor H-related protein (160, 161), which serve to ward off infection, as well as laminin (159, 162). In another study, CRASP-1 bound PLG and cleaved chromogenic substrate S-2251 (161).

Increasing evidence shows that the affinity for host PLG and components of the complement system is also a unifying characteristic among various *Borrelia* species other than *B. burgdorferi sensu stricto*. *B. afzelii* and *B. spielmanii* (126) as well as *B. burgdorferi* CspA (CRASP-1) bound factor H and PLG, concurrently and once the PLG was activated by uPA, it was able to partially degrade central complement protein C3b (163). Additionally, relapsing fever spirochete *B. hermsii* expresses a novel form of CRASP-1 (designated BhCRASP-1), bound to complement regulators as well as PLG (163). Surface lipoprotein BpcA from *B. parkeri* bound factor H and factor H-related protein 1 as well as PLG, degraded S-2251 and fibrinogen, and conferred resistance to complement-mediated killing. The BpcA homologue from *B. turicatae* failed to bind complement system regulators, while its ability to bind PLG was preserved (164). Surface protein FhbA, a 20.5-kDa lipoprotein from *B. hermsii*, bound factor H and PLG, identifying it as an important molecule in RF pathogenesis (119, 165). Finally, *B. miyamotoi*, the RF agent of miyamotoi disease, a Lyme disease-like syndrome, expresses a surface-exposed protein, CbiA, that exhibits PLG-binding properties, and the bound PLG can be converted to active plasmin capable of cleaving fibrinogen (145).

The enzyme enolase is a well-known component of the glycolytic pathway which catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. In some bacteria, enolase is also a surface-exposed protein that functions as a PLG receptor (166–169). Accumulating evidence points to a similar role in *B. burgdorferi* (170–172). *B. burgdorferi* enolase, in addition to its cytoplasmic function in glycolysis, would also

have to be exported to the spirochetal outer membrane for it to bind plasmin(ogen) and facilitate extracellular proteolysis needed for spirochetal spread across tissue barriers. Experimental evidence lends credence to this idea. *B. burgdorferi* enolase has been shown, through immunoelectron microscopic analysis and proteolytic digestion, to be in the outer membrane fraction as well as in the cytoplasm (173), surface exposed (170, 172), and in outer membrane vesicles (OMVs) shed by *B. burgdorferi* during culture (171). The enolase in the OMV is accessible to proteolytic degradation by proteinase K, providing evidence for its surface exposure in OMVs. Moreover, the importance of enolase in infection is implied by its higher production under conditions that mimic the conditions in the mammalian host (171). Taken together, these findings suggest a moonlighting role for enolase in *B. burgdorferi* by virtue of its role in stabilizing cell-bound or extracellular OMV-bound plasmin in addition to its canonical role in glycolysis. The binding of PLG to bacterial enolases is not restricted to *Borrelia*. *Streptococcus canis* enolase binds to both human and canine plasminogen and facilitates degradation of fibrin after activation with host-derived uPA (174). *Leptospira interrogans* binds PLG in what appears to be a unique manner, whereby the enolase is secreted, binds PLG, and reassociates with the organism (172).

Other *B. burgdorferi* outer surface proteins have been identified as interacting with PLG. Assays using radiolabeled human PLG to bind *B. burgdorferi* identified two prominent 70- and 30-kDa PLG-binding outer membrane proteins, of which, the 30-kDa band was found to be OspA (152). The 70-kDa band, termed BPBP, adsorbed approximately 10 times more label than OspA (175). An interesting and unexpected finding was that there was seemingly no difference between low-passage-number (infectious) and high-passage-number strains in the binding of PLG (152, 175). Genetic alignments showed BPBP to be an ortholog of a 60-kDa protein found in *B. coriaceae* (66% identity, 80% similarity). This protein was antigenic in infected patients (175). In another study, recombinant hypothetical protein BBA70 bound PLG in a dose-dependent manner, and the bound plasmin degraded chromogenic substrate S-2251 and natural substrate fibrinogen as well as complement system components C3b and C5 (150).

### Interaction of *Borrelia* with Other PAS Components

As mentioned earlier, *Borrelia* spp. interact with each of the components of the PAS (Fig. 3). *Borrelia* spp. interact with the matrix metalloprotease system (Fig. 3) by inducing the production of these proteases from a diverse group of cells. The level of interaction of *Borrelia* and all the components of the PAS is thorough and meets all of its physiological requirements. Lyme and relapsing fever *Borrelia* spp. stimulated human peripheral blood monocytes and neutrophils to release pro-matrix metalloproteinase-9 (pro-MMP-9; gelatinase B) and active matrix metalloproteinase-1 (MMP-1; collagenase-1). U937 cells and human keratinocyte cells released pro-MMP-9, and plasmin stabilized on the surface of the Lyme disease spirochete activated pro-MMP-9 to its active form. This active form was also observed in the plasma of mice infected with a relapsing fever *Borrelia*. These results showed that *Borrelia* can upregulate MMPs and mediate an activation cascade initiated by plasmin bound to the microbial surface. *B. burgdorferi*-bound plasmin triggered the conversion of pro-matrix metalloproteases (MMPs) to active MMP-9 (type IV collagenase), establishing an indirect role for plasmin in collagen degradation (176).

Attempts at working out the mechanisms for production of MMPs by the spirochetes is tied to proinflammatory pathways. Secretion of MMP-9 by *B. burgdorferi* is selectively induced through Toll-like receptor (TLR) 2 in human and murine monocytic cells (176). Secretion of MMP-1 was shown to be stimulated through a pathway other than TLR2 (177). *B. burgdorferi* utilizes several signal transduction pathways for the production of MMPs from human joint cells (178). *B. burgdorferi* induced transcription of MMP-1, -3, -13, and -19 from primary human chondrocyte cells (178). Transcription of MMP-10 and tissue inhibitor of MMP-1 were also elevated but without concurrent protein expression. The synovial fluid levels of MMPs in patients matched the experimental results. In contrast, infection of mice with *B. burgdorferi* only induced production of

MMP-3 and MMP-19 (179). *B. burgdorferi* binds to integrin $\alpha(3)\beta(1)$, and binding of this complex induces production of matrix metalloproteinases in primary human chondrocyte cells (180).

MMPs have also been detected in clinical specimens, emphasizing their role in Lyme disease pathogenesis. Not surprisingly, MMPs have been associated with the skin, neurological, and joint manifestations. MMP-9 was detected in the cerebrospinal fluid (CSF) of patients with neuroborreliosis, and infiltrating macrophages were thought to be a possible source of the MMP-9 increase (181). Levels of MMP-1 and MMP-3 in synovial fluid were higher in untreated Lyme arthritis patients. In contrast, MMP-8 and MMP-9 were elevated in patients with antibiotic-resistant arthritis, illustrating the diversity of stimulation pathways depending on disease stage (182). MMP-9 was upregulated in erythema migrans lesions (183). Possible mechanisms for MMP induction have been proposed as well. Levels of MMP-9 and soluble CD14 were markedly elevated in serum from patients and were also upregulated in U937 cells by *B. burgdorferi* in a time- and concentration-dependent manner. When fibroblasts incubated with supernatants from U937 cells were exposed to *B. burgdorferi* or recombinant CD14, the expression of MMP-9 was significantly increased (184).

As previously stated, the *Borrelia* spp. interact with all the components of the PAS (Fig. 3). In coculture experiments with monocytic cells, *B. burgdorferi*, as well as various spirochetal components, induced the expression of cellular uPA receptor (uPAR) as well as the release of a soluble form. The upregulation of uPAR by *B. burgdorferi* was concomitant with an increase in uPAR mRNA. The mechanism for soluble uPAR release and its role were not apparent in this study (185). In an ensuing study with monocytic cells, combined functional blockade of CD14 and TLR2 results in significant, but not total, inhibition of *B. burgdorferi*-dependent uPAR induction. It was concluded that signaling for monocytic cell uPAR expression mediated by *B. burgdorferi* proceeds with CD14 and TLR2 as partial contributors. That part, under the control of CD14 and TLR2, represents a link between the host PLG activation and innate immunity systems (186).

### Borrowed Proteolysis and Host Defense

The most accepted (perhaps the only) outcome of having active plasmin on the surface of *Borrelia* is increased invasiveness. Resulting proteolysis of matrix proteins and connective tissue by bound plasmin can assist this highly motile organism in invading tissues. Likewise, proteolysis of the external milieu of the spirochete by bound plasmin could be an important means for nutrient acquisition, and this is an area that merits future investigation.

What if PLG binding followed by uPA activation was a host defense mechanism against the bacteria that utilize the PAS? There are scant but tantalizing indications that this could be the case. One such early indication comes from the stimulation of uPAR production by *B. burgdorferi* (185, 186). In a far-reaching study, uPAR knockout mice had significantly higher numbers of *B. burgdorferi* than the controls (187). These mice also displayed impaired phagocytic capacity. In uPAR knockout mice backcrossed to a C3H/HeN background (the preferred inbred mouse strain for laboratory infection), the higher numbers of spirochetes were associated with more severe carditis and increased local TLR2 and interleukin 1 beta (IL-1$\beta$) expression. This study concluded that in *B. burgdorferi* infection, uPAR is required for phagocytosis and adequate eradication of the spirochete from the heart by a mechanism that is independent of the binding of uPAR to uPA or its role in the fibrinolytic system (187, 188). Moreover, the recently discovered PLG receptor on the surfaces of cells of the innate immune system (189) is important in the PLG-dependent regulation of the migration, invasion, and recruitment of macrophages and inflammatory monocytes (190, 191). This is an expanding area of investigation (188) that could have an impact on the host defense against PLG-bound bacteria.

## CONCLUDING REMARKS

In this review, we have considered the protease contingent of *Borrelia*, from annotation in the genome to the research on specific proteases of these spirochetes. The

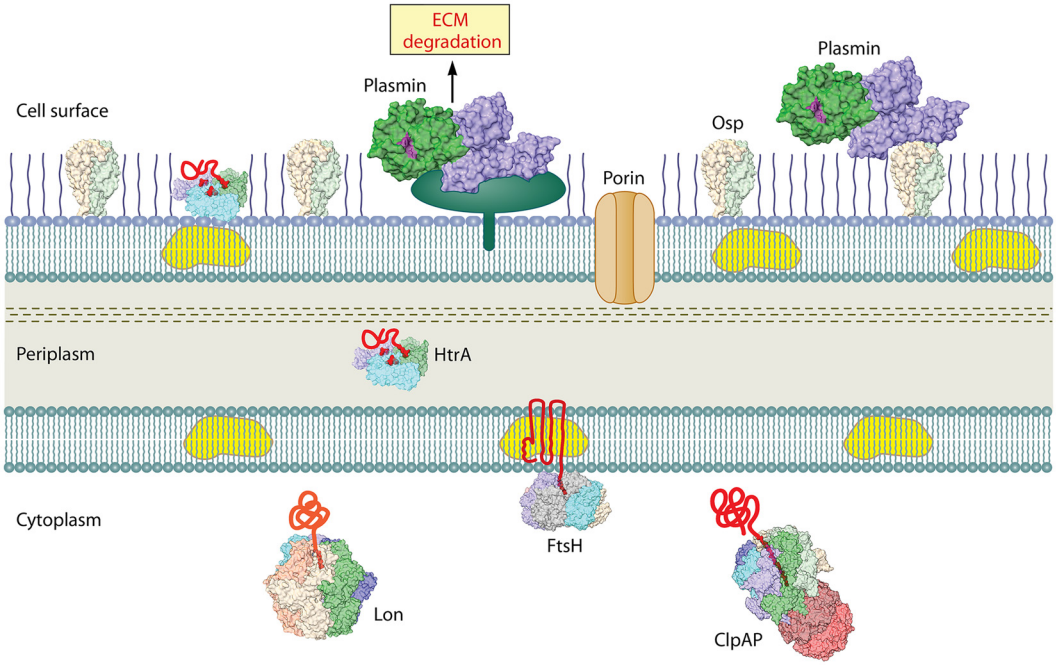


**FIG 4** A schematic overview of the key endogenous and borrowed proteases *Borrelia* spp. use to remodel their proteomes as they move from one stage, tissue, or host to another. Major cytoplasmic and inner membrane proteases, Lon, ClpAP (as a representative of the Clp protease systems ClpXP, ClpYQ, and ClpCP), and FtsH are shown. There are bound substrates highlighted in red. The lone periplasmic protease HtrA, which is also secreted, is shown in the periplasmic space and associated with the cell surface. The yellow ovals represent cholesterol-rich lipid rafts, which are of functional importance and exist in both the inner and outer membranes of *Borrelia*. The borrowed plasmin proteolytic system is shown bound to *Borrelia* outer surface proteins (Osp), and additional cell surface receptors, shown as a green oval. The 5 kringle domains of plasmin are colored purple, and its protease domain is light green. The periplasmic flagella are not shown.

review has shown some unique features of the proteolytic arsenal (Fig. 4) and the realization that *Borrelia* spp. (both RF and Lyme species) have dedicated a large percentage of their genomes to proteolysis. The proteolytic activities of secreted HtrA have led to new paradigms of pathogenesis, and we reviewed this aspect in the context of other bacteria. The interactions of *Borrelia* with all the components of the plasminogen activation system is an example of pathogens appropriating host proteolytic activities. Borrowed proteolysis and its role in pathogenesis, not only by infection with *Borrelia* but also by infection with other bacteria, could be viewed as a mechanism of host defense. One of the major reasons for writing this review was to consider the overwhelming evidence of the utilization of the PAS by *Borrelia*. There is no other mammalian system so intertwined with a group of pathogens such as the RF and LD *Borrelia* spp. Moreover, the evidence for the utilization of the PAS stems from experimental *in vitro* studies to *in vivo* mouse model experiments and to actual studies with patients with borrelioses. Despite this, there is much that needs to be done, as surprisingly little is known about the targets of the spirochetal harbored proteases, their temporal regulation, and their unique mechanisms of action. Likewise, a reassessment of borrowed host proteolysis could reveal surprising consequences for pathogenesis.

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